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Abstract: Obese or overweight people exceed one-third of the global population and obesity along with diabetes mellitus consist basic components of metabolic syndrome, both of which are known cardio-cerebrovascular risk factors with detrimental consequences. These data signify the pandemic character of obesity and the necessity for effective treatments. Substantial advances have been accomplished in pre-clinical research of obesity by using animal models, which mimic the human disease. In particular, rodent models have been widely used for many decades with success for the elucidation of the pathophysiology of obesity, since they share physiological and genetic components with humans and appear advantageous in their husbandry. The most representative rodents include the laboratory mouse and rat. Within this review, we attempted to consolidate the most widely used mice and rat models of obesity and highlight their strengths as well as weaknesses in a critical way. Our aim was to bridge the gap between laboratory facilities and patient's bed and help the researcher find the appropriate animal model for his/her obesity research. This tactful selection of the appropriate model of obesity may offer more translational the derived results.. In this regard, we included, the main diet induced models, the chemical/mechanical ones as well as a selection of monogenic or polygenic models.

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Rodent models of obesity

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Abstract

Obese or overweight people exceed one-third of the global population and obesity along with diabetes mellitus consist basic components of metabolic syndrome, both of which are known cardio-cerebrovascular risk factors with detrimental consequences. These data signify the pandemic character of obesity and the necessity for effective treatments. Substantial advances have been accomplished in preclinical research of obesity by using animal models, which mimic the human disease. In particular, rodent models have been widely used for many decades with success for the elucidation of the pathophysiology of obesity, since they share physiological and genetic components with humans and appear advantageous in their husbandry. The most representative rodents include the laboratory mouse and rat. Within this review, we attempted to consolidate the most widely used mice and rat models of obesity and highlight their strengths as well as weaknesses in a critical way. Our aim was to bridge the gap between laboratory facilities and patient's bed and help the researcher find the appropriate animal model for his/her obesity research. This tactful selection of the appropriate model of obesity may offer more translational the derived results.. In this regard, we included, the main diet induced models, the chemical/mechanical ones as well as a selection of monogenic or polygenic models.

Introduction

Obesity is characterized by increased fat mass in body and caused by a prolonged misbalance of energy intake and energy expenditure [1–3]. The expansion of adipose tissue is the final result of both environmental factors (including hypercaloric food intake and physical inactivity) and genetic predisposition [4]. Hereditability is estimated to range from 40 – 70% [5].

Obesity consists a global substantial clinical and economical burden reaching nowadays pandemic proportions, since about 1.5 billion adults are overweight and, among them, it is estimated that 200 million men and 300 million women are obese [6]. Obesity has also an increasing prevalence in childhood, adolescence and women of reproductive age, both in the developed and developing countries [7,8].

Obesity related to metabolic syndrome (MetS) represents a principal risk factor for diseases and disability [9]; it serves as substrate and basic component of MetS and hosts many comorbidities including hypertension, hyperlipidemia, diabetes mellitus type 2 (T2DM), chronic kidney disease, cardiovascular disease (CVD), osteoarthritis, malignancies and nonalcoholic fatty liver disease (NAFLD) [10,11]. A subcategory of obesity without MetS, referred to as metabolically healthy obesity has yielded contradictory estimates of association with CVD [12].

A number of animal models have been used to study the obese response to various forms of disease. In this regard, preclinical animal models have been used successfully over many decades for the purposes of basic research, so as the complex pathogenetic mechanisms of diseases are elucidated. Two main types of obese animal models are used: monogenic (mutation linked to only one gene) and polygenic [diet-induced obesity (DIO)] [13,14].

Generally, monogenic models have commonly used a mutation in the leptin pathway, though it has been revealed that the single manipulation of almost 250 different genes in mice can induce obesity [3]. In DIO models, obesity is often produced by feeding animals a

hypercaloric diet. Two frequent variants include the high-fat diet (HFD) [15], which induces obesity owing to its high energy density, and the cafeteria diet, which stimulates hyperphagia (and consequent obesity) owing to its palatability [3]. Other less common models of obesity include those produced by chemical or surgical approaches [3].

A wide variety of animal models are reported in numerous publications, from worm *Caenorhabditis elegans* and fly *Drosophila melanogaster* to Zebrafish and mammals (non-human primates or not). Rodents have dominated for a long time in biomedical research and are represented mainly by the laboratory mouse (*Mus musculus*) and rat (*Rattus norvegicus domesticus*) [16]. Other species being used include pigs, sheep and even unusual ones including seals and bats [3]. Mice and rats are regarded as the ideal animal models for obesity research and preclinical research generally and are preferred over other animal models, since they share many anatomic, physiologic as well as genetic similarities with human [17]. Noteworthy, they have along with human about 30,000 genes, 95% of them are common in these three species [17]. Furthermore, rodents are characterized by a small body size and rapid reproduction, which constitutes their husbandry easy and inexpensive.

The contribution of mice and rats to obesity and diabetes research is important. We hereby demonstrate some representative examples. The revolutionary discovery of adipocyte-derived cytokine hormone leptin [18] and its receptor [19,20] was a consequence of characterization of the underlying genetic defect in mutant mice (both *db/db* and *ob/ob*); the *ob/ob* mouse, one of the monogenic animals most used in the study of obesity and MetS, does not produce leptin, but is still sensitive to it [21]. Likewise, the substantial elucidation of the role of melanocyte stimulating hormone and its associated agouti-related peptide [22,23] signaling via the melanocortin-4 receptor [24,25] was also performed in mice. Of note, the aforementioned genes as well as the one of leptin, are all known etiologies for monogenic obesity of human [2]. Furthermore, the discovery of another novel central key-player regulator of energy balance, ghrelin, took place in rats [26].

In this review, we summarize the main rodent models of obesity, highlighting respectively the advantages as well as limitations characterizing each model. We followed an etiologic classification, distinguishing also between the species and genetic substrate (Figure 1); obesity models can be developed easily in a chemical/mechanical way, by modifying the diet (DIO), or by modifying genome (the so-called genetically engineered models, GEM). A synopsis of all discussed models can be found in enclosed Table 1. We referred to some models, whose use is declining, mainly for historical reasons. Nonetheless, an extensive description of all ever-used rodent models exceeds the scope of the present review.

Diet-induced (caloric excess) rodent models of obesity

DIO belongs to the traditional and widely used rodent models mimicking human obesity [27–29]. Animals are given typically high caloric food (rich in sugar and/or fats) *ad libidum* and the progression to obesity and diabetes development is slow and recapitulates in a satisfactory manner the corresponding human pathology, in which fat is slowly accumulated, mainly due to excess of energy and insufficient expenditure [28,29]. Although there is a lack of standardized, classic DIO, many labs use defined HFD (see also Table 2), which contain 45-60% calories from animal source fat (lard) [27,29], the latter having a more pronounced effect on insulin resistance (IR) and adiposity than fat of vegetable origin [30]. Impaired insulin tolerance can be seen as early as one week after the start of diet and weight gain from the second to forth week, whereas a peak of the body weight can be obtained about 16-20 weeks from the beginning of HFD. Mice weigh 20-30% more than those fed on a standardized balanced diet (controls) [27]. Nevertheless, studies with DIO are often more expensive and as mentioned time consuming and there is also a plethora of additional factors, which might limit the interpretation of results [28,29]. A representative paradigm is the varying susceptibility among different murine strains, due to particularities of their genetic substrate. C57BL/6J, derived from

the same parental C57BL/6 strain, is a typical mouse strain being used for obesity and IR research. Other strains, like A/J and SWR/J are more resistant, thereby less appropriate for obesity and diabetes studies. Sprague-Dawley and Wistar rats, typically used as DIO/Mets [31], acquire this disorder more easily, signifying that the genetic basis is essential in body weight gain [32]. Regarding the latter polygenic animal strain, it has also been used for studies of DIO demonstrating an increase in body weight [33], though the results on changes in insulinemia are conflicting [34,35]. Remarkably, the female Wistar rat model has also been introduced as a DIO model during pregnancy, as a gestational obesity model and as a model for evaluation of offsprings born by obese mothers. The usage of hypercaloric diet during the gestational and lactation periods of Wistar rats appears to modify the obesity phenotype in the obese mothers offspring, indicating the significance of maternal nutrition [33].

Another parameter, which should be considered upon planning the protocol of an experiment, is the gender selection of the rodents. It is known that male mice develop obesity more quickly and to a greater extent than the female ones. This sex preference affects also the accompanying IR and diabetes onset [28].

Nonetheless, it should be emphasized that rodents as model for the study of pancreatic islets are less appropriate, as their anatomic architecture deviates [28]. The translational value of this murine particularity regarding sex reflects partially to human patients with men being more prone to diabetes onset, but women more prone to obesity.

This phenotypic sexual difference can be interpreted, due to different gonadal hormones (estradiol/progesterone versus testosterone), which impacts the topographical distribution of fat [28]. Particularly, it is known that downregulation of the tumor suppressor phosphatase and tensin homolog (PTEN) leads to retention of insulin sensitivity with accompanied higher adiposity in women compared to men[36]. Additionally, AEBP1 is a transcriptional repressor, which serves as regulator of PTEN expression in a negative way. It has been found to play a key-role in modulation of adiposity via fat-cell proliferation and is implicated in a gender-specific

susceptibility to diet-induced obesity by the estrogen signaling pathway[37]. Moreover, HFD-fed AEBP1 females exhibit over-induction of AEBP1 and a noticeable reduction of PTEN level with concomitant hyper-activation of the survival signal in adipose tissue[37].

An additional parameter, which has to be taken into account for murine experiments, is the age. For instance, C57BL/6J strain reaches a maximal body weight at nine months after birth and and C57BL/6J mice 22 months old appear reduced lean mass and increased fat mass compared with young 3-month-old. This simulates analogously the observed phenomenon in humans, where a fat redistribution takes place at 30 years of life and a peak of body weight is anticipated for both genders at about 55 years. Furthermore, epigenetic inheritance of diet-induced obesity is well described and plays an important role in performed experiments [28]. Interestingly, many studies have demonstrated the effects of an *in utero* HFD on offspring weight, regardless of the animal model; these observations have been replicated in human MetS [38–40]. The exact molecular mechanism is not well defined, although DNA methylation and histone modifications seem to play a key role [38].

The modern diet in humans is frequently comprised of high level of fats and carbohydrates. Rodent relative data are based on these diets, though there is variation in the quantity of components introduced and in their source, which might alter the animals' phenotype [32]. It is worthy, however, that, although many rodent diets are close enough in composition to the human ones of modern western societies, the regime of feeding *ad libidum* with the identical meal throughout the light dark-cycle (circadian rhythm), more or less for many weeks is monotonous and let the animals without choice. Such a simplified approach might be attractive for the performers of an experiment, although does not reflect satisfactory the habits of human in the real world, where a wide variety of meals is available [29]. In order to cope with this problem, several custom diets have been introduced, the most common of which are briefly reported below.

Cafeteria (supermarket) diets

'Cafeteria' diets (CAF-Ds) provide to animals a mixture of savory and sweet, high fat and/or high sugar solid nutrition emanating from human larder. They appeal as attractive models of modern human obesogenic diets [29]. CAF-D-induced obesity is the result of hyperphagia, which is only partially compensated by increased energy expenditure. This phenomenon is feasible particular in diet-induced thermogenesis, once sympathetic activation of brown fat takes place. Hyperphagia of cafeteria diets is explained by increase of both meal portion and meal frequency. This contrasts with overeating of palatable diets with no choice of food, which mainly influences meal size [41]. Nevertheless, the aforementioned options are used less frequently than diets of a single-source fat, due to the existing of logistical constraints and sceptism over the heterogeneity and lacking of standardization, making calculations of energy and macronutrient intake rather difficult [29]. Due to these concerns, CAF-D regime has been lately revised. The so-called "junk food" diet offers a choice of snacks / processed food apart from a lard/chow mix and has been introduced to feed adult Wistar rats (both genders) [42], to rat dams before being mated and until their offspring are weaned, and to their offspring as well [43]. This regime resulted in increases in body fat mass in all groups. Moreover, perinatal exposure of offspring to high-sugar and high-fat diets increased their intake of lipids the time of weaning [29]. Of note, CAF-D-related obesity seems to induce an increased oxidative damage in white adipose tissue affecting tissue homeostasis and oxidative stress (OS) drives activation of inflammatory kinases that can disturb insulin signaling resulting in glucose intolerance and diabetes [44]. Moreover, the noticeable microbiota dysbiosis observed in CAF-fed rats might result from the presence of several additives of the CAF diet, or even a absence of essential vitamins and minerals [45]. The gut microbiota dysbiosis-derived products provoke low-grade inflammatory activation of tissue-resident macrophages and contribute to metabolic and degenerative disorders, including obesity diabetes and MetS [46].

Regarding the mentioned OS, interestingly, recent data indicate that the therapeutic effects of resveratrol against hepatic steatosis of HFD obese male C57BL/6 mice are attributed to the decrease of hepatic OS, inflammation and free fatty acid uptake [47]**.** Likewise**,** *Cordyceps militaris* with its anti-obesity actions can be inhibited endoplasmic reticulum (ER) stress and ER stress-induced apoptosis in the hepatic steatosis of this HFD-induced obesity model [48].

Fat or sugar choice diets

This sort of diets is a simplification of a CAF-D, which aims at overcoming the previously described limitations [29]. For instance, la Fleur et al. proposed a relevant rat model [49], in which the free-choice of "high-fat, high-sugar" diet allows rodents to choose between a dish of saturated fat, a bottle of 30% sucrose, and standard chow. Animals were persistently hyperphagic (eating to excess) and became obese compared to those fed a no-choice pre-mix off at, sugar and chow, which induced only transient overeating. Increased caloric intake in the free-choice group was the result of an increase in the number of meals due to drinking of sugary liquid without any modification of meal size [49]. This obesity model is attractive due to inducing the desired overconsumption of calories by offering a free choice of diet depending on appetite of animals [29].

Sugar based diets, namely the ones containing simple carbohydrates can be divided to fructose-enriched (fruit monosaccharide) and sucrose-enriched (the common table sugar, containing one molecule of monosaccharide glucose and one of fructose) [31]. Fructose high levels in mice mimic the human diet and, when associated with high fat content, promote weight gain, abdominal fat, hyperglycemia and hyperinsulinemia [50]. Fructose appears to be important in obesity and MetS development, because this sugar induces not only IR, but also leptin resistance, thereby leading to weight gain [37,51,52]. Fat based diets might use either as source plant-derived oils (for instance olive oil, corn or safflower) or animal-derived oils (i.e. lard or beef tallow)[31].

Meal feeding

There are different models of meal feeding, depending on the methodology used in each study and its purpose. Their role in obesity studies remains limited, as specific feeding patterns are involved and resulted obesity and adiposity are affected by multiple variances. On the other hand, senses before and after the meal and the central nervous system (CNS) reactions have been examined by these techniques, using the advantage of conventionalizing the normal physiological status around the meal [53]. The most recognizable example of that method is the *ad libitum* model, which simulates to the natural situation of rodents, offering to the subjects an unlimited access to a specific HFD. More functional and commonly used alternatives include predetermined periods of food provisioning; disposal of the meals at specific intervals and for limited time along day [29]. Recent data indicate that time-restricted feeding entrains diurnal rhythmicity in vagal afferent mechanosensitivity in both lean and HFD-induced obese mice and inhibits the loss of rhythmicity in HFD-induced obesity, thereby having implications for the development of relative therapeutic strategies against obesity [54].

Binge-type feeding

Based on the principle that eating requirement is the resultant of daily habits, social environment, or convenience, this method is often used to study and control eating behavior, rather than obesity and metabolic disorders [42,55]. The mainstay of binge-type feeding is the flavorful meal –provided in scheduled intervals simultaneously with a basic diet. This "rewarding" pattern could result in obesity in models with greater alterations in limbic and motivational circuits in response to palatable food [42,55,56].

The aforementioned possibility led to proposition of a classification based on rats' susceptibility to binge-type diet. Binge eating prone (BEP) rodents gorge more than twice of

the palatable food instead to binge eating resistant (BER) rats. Apparently, the resulted obesity is more common to the first subgroup [57].

From a clinical point of view, excessive consumption of highly-palatable and energy-dense foods is commonly observed in patients with eating disorders (binge eating disorder [BED] and bulimia nervosa [BN]) and in some obese patients [58]; binge-eating behavior is a main symptom in several eating pathologies but the neurobiological triggering factors of these disorders remain elusive [59].

Impact of maternal diet on offspring

Maternal diet effect on offspring remains a challenging issue in obesity and metabolic disorders. Brain programming, including hypothalamus development, is known as a key factor implicated in appetite regulation and energy homeostasis and follows common pathways among mammals [60]. Nevertheless, postpartum hypothalamic maturation in rodents provides an important advantage to study the impact of maternal feeding to the hormonal stimulation and neuroregulation [61]. The role of hormones, such as leptin and ghrelin, in hypothalamus formation could illuminate the importance of maternal diet and broaden the treatment prospects [62,63]. In a recent meta-analysis, Ribaroff et al. included 171 animal studies of maternal diet models, underlying its impact to the offspring bodyweight and the overall metabolic profile, and suggesting the high maternal carbohydrate consumption as a possible causative factor [64]. Moreover, recent evidence, by using the above mentioned C57BL/6 mouse model, indicates an augmented vulnerability of male offspring to maternal obesity-produced alterations in chromatin remodeling processes that regulate gene expression in the developing hippocampus, thereby contributing to our knowledge of how early life nutrition affects the brain epigenome offspring; *in utero* exposure to maternal HFD-induced obesity modifies oxytocin receptor (Oxtr) gene expression and histone binding at the Oxtr promoter in offspring hippocampus in a

sexually dimorphic manner; and in response to HFD, Oxtr transcription augmented and H3K9Ac (an active histone mark) binding was enriched at the Oxtr promoter in hippocampus male offspring [65].

In conclusion, DIO models seem to be close to the mechanisms that induce obesity and MetS in humans [32]; these models mimic human obesity, and thus would be used for preclinical testing of the role of diet, etiology, pathophysiology and therapeutic interventions. Nevertheless, the results of the studies are inconsistent, mostly in relation to the diet compositions and the type of model used [32,66]. Diverse strains of animals exhibit variable responses to these diets, with certain being prone and others being more resistant to obesity development [41]. Because of this and the difficulty of long-term feeding with a certain diet, morbid obesity is not easy to replicate in DIO models.

Chemical (pharmacological) models of obesity

These models were originally proposed for the study of type 1 diabetes mellitus (T1DM). Well described diabetogenic agents, which are structural analogues of glucose include alloxan and streptozotocin and have been used for the selective destruction of pancreatic β-cells [27,28,67]. For induction of obese phenotype, however, a combination with a HFD/HSD is mandatory. Pathogenetically, the intracellular entry is mediated via glucose transporter type 2 (GLUT-2) [27,68]. GLUT-2 is however, also expressed in kidney and liver, thus the administration of the above substances is nephrotoxic and hepatotoxic or even tumorigenic [27,28]. Induction of diabetes is mediated by selective necrosis of pro-beta islet cells, which are the insulin-secretory pancreatic cells[27]. Alloxan leads to beta-cell dysfunction and death by inhibiting glucokinase, with induces the production of reactive oxygen species[27]. Streptozotocin mediates mechanistically its action by alkylating or breaking DNA strands, which has as consequence

the increase in the activity of poly-ADP-ribose synthetase, finally resulting in energy deprivation and cellular death[69].

Systemic toxicity of both streptozotocin and alloxan are typically seen within the first two weeks after diabetes induction. The latter displays a particularly narrow window of safety between the diabetogenic and lethal dose and deaths are often a result of diabetic ketoacidosis and/or renal or hepatic failure[70]. Alloxan nephrotoxicity especially is a consequence of the lipophilic derivatives after systemic administration and can be so severe that it leads to fatal renal failure in the animals even before diabetes can develop[71]. Likewise, the cell toxicity of streptozotocin is not limited to beta cells, but also induces direct acute damage to the kidneys [72] as well as the liver and intestine[73].

Streptozotocin is generally preferred over alloxan, as it has a better profile, i.e., more stability and less toxicity[28]. A complete depletion of all β-cells of pancreas mimics T1DM, which is inappropriate for the study of obesity or MetS. Nevertheless, a partial destruction of some β-cells by inoculating low doses of streptozotocin can resemble human T2DM and obesity when a special aforementioned "junk" diet is combined. A single or even multiple shot(s) of 25 – 70 mg/kg are given depending on the customized protocol (in which the age of rodent and the species-strain are primarily considered) and preferably a high fat/"western" diet is added, so as the slow progression of T2DM with obesity can be achieved [28,68].

To the chemical models of obesity belongs also the so-called aurothioglucose-induced hypothalamic obesity although there are limitations, which constrain its usage[69]. Aurothiglucose, also known as goldthioglucose (GTG), can be administered intraperitoneally

for the introduction of diabetes and obesity in a dosage 150-350mg/kg body weight. Onset of the desired phenotype is slow and may take up to 20 weeks after injection. Pathogenetically, the substance reaches neural cells of ventromedial hypothalamus and induces necrosis, which leads to hyperphagia associated obesity. Specifically, GTG induces an region of necrosis of ventromedial hypothalamic (VMH) areas, apparently by primarily targeting glucose-sensitive neurons[74]. The onset and extent of VMH lesions are dose-dependent and lesions become histologically obvious after 12 hours[75]. GTG-treated mice develop hyperphagia, in which glucose administration induces no satiety effect[76]. These mice are also characterized by increased body lipid and hepatic lipogenesis and triglyceride secretion as well as increased adipose tissue lipogenesis and decreased glucose metabolism in muscles. These changes appear similarities to the later discussed genetically obese mice (*ob/ob*). Nevertheless, the main disadvantages include the long time-frame to develop obesity/diabetes and the exceptionally high rates of mortality after aurothioglucose injection[69]. The GTG mouse model is still used, for instance, to determine the mechanism of agents improving IR and preventing obesity[77] and to learning the role of microRNA in insulin-resistant conditions, such as obesity or type 2 diabetes mellitus [78].

Other, less used, pharmacological agents for induction of obesity include cyproheptadine, a first generation antihistamine, clonidine, an adrenergic α2 receptor agonist, and chlordiazepoxide, a benzodiazepine [79].

Important to note, there is a connection between the developmental exposure to a number of endocrine disrupting chemicals (EDCs) and diseases outcomes across the lifespan in animal models including obesity, TDM2 and fatty liver disease, thereby signifying the development of new biomarkers of exposure and disease outcomes as well as intervention and prevention studies [80]. In this respect, the term “obesogen” refers to a chemical that could result in increased weight gain either directly through effects on fat cell development or indirectly through effects on other endocrine organs or the brain that could lead to augmented weight gain

[81]. Currently, it is clear that T2DM and NAFLD can also be programmed *in utero* and early life by exposure to environmental chemicals in animal models [80,82,83]. An examination of environmental chemicals that caused obesity, T2DM or fatty liver diseases showed that exposure to many of the same chemicals led to all three diseases. Besides, toxicology and epidemiology studies have suggested the involvement of diverse EDCs in an increasing number of metabolic disorders, including IR and IR-related co morbidities, such as obesity, T2DM, NAFLD and polycystic ovary syndrome, suggesting that EDCs could play an important role in the pathogenesis of these disorders [84]. Thus, it has been proposed that they should be called metabolism disrupting chemicals (MDCs or metabolism disruptors) as they can lead to MetS. These include bisphenol A, DDT/DDE, tributyltin, smoking/nicotine, arsenic and it is likely this list will expand as more studies examine more endpoints related to metabolic diseases [80]. The prevention of this field is an emerging area with enormous potential that only emerged in the last decade. There are a variety of approaches to mitigate or prevent these disorders from exposure to environmental chemicals.

The advantages of pharmacological models include: i. they are comparatively cheaper, easier to develop and maintain. ii. selective loss of pancreatic β -cells with streptozotocin or alloxan leaves pancreatic α- and δ-cells intact. iii. Residual insulin secretion constitutes the rodents live long-term without being depended on insulin treatment and iv. Ketosis and resulting mortality are not common [69]. Basic disadvantages of the pharmacological models include: i. possible toxicity at rest organs of the body. ii. Changes in P450 isozymes activity in the liver, kidney, lung, intestines, testis and brain have been reported after administration of alloxan or streptozotocin, which may affect the efficacy of the investigated medications, if they are metabolized at the same isoenzymes. iii. Study of diabetes is less optimal, since hyperglycaemia varies and diabetes is not stable [69,85].

Surgical (mechanical) models of obesity

This category consists one of the oldest being proposed for inducing rodent obesity and includes hypothalamic, ovarian and brown fat surgical interventions [41].

Lesion of the ventromedial hypothalamus (VMH)

Hypothalamic lesions were incriminated to induce obesity in the middle of 19th century [86]. The two widely used techniques to induce a VMH lesion in rats include the subcutaneous or intraperitoneal administration of monosodium glutamate (MSG) and electrical ablation [87].

The most widely accepted mechanism of obesity via VMH lesions is the imbalance between sympathetic and parasympathetic stimulations [88–90]. Vagal hyperactivity in VMH lesion models is associated with hyperinsulinemia, pancreatic islet cell hyperplasia, sensitive to streptozotocin and higher serum triglycerides [8,41,91]. The plethora of receptors of weight regulating molecules, including leptin and melanocortin, in VMH and the comprehension of their pathophysiology tend to illuminate the exact effect of VMH lesions on obesity [91]. Recent data indicate that carnitine palmitoyltransferase 1C (CPT1C), implicated in central regulation of energy, is essential for the activation of brown adipose tissue (BAT) thermogenesis driven by leptin, HFD exposure, and AMPK inhibition within the VMH, thereby underscoring the significance of uncoupling protein 1 (UCP1)C in the activation of BAT thermogenesis to counteract diet-produced obesity [92].

Lesion of the hypothalamic paraventricular nucleus (PVN)

PVN is a key interface between the endocrine, autonomic and somatomotor systems that influence feeding behavior and energy metabolism [93]. Food overconsumption and subsequent

obesity of PVN lesion animal models follows the same pattern with the VMH lesions ones, however, lacks their manifestations [41,89]. PVN neurons express a variety of weight modulating molecules and receptors [29,91]. Maternal HFD during pregnancy and lactation seems to modify orexigenic mediators expressed by PVN neural cells in offsprings [29].

Shi et al. suggested that PVN possesses an additional role to MetS**,** regulating the neurocircuitry of adipose afferent reflex in rats and subsequently the systematic blood pressure and hypertension [94]. Moreover, recent data indicate that damage to Sim1 neurons (family BHLH Transcription Factor 1 (Sim1) neurons essential to control energy homeostasis) of the PVN is a shared feature of exposure to HFD in mice of both sexes [95]; and caffeine, an antagonist of the adenosine receptor A_1R, used as a dietary supplement to reduce body weight, reduces A_1Rs expressed on PVN oxytocin neurons to negatively regulate energy balance in DIO mice [96].

Lesion of the arcuate nucleus (ARC)

Surgical demarcation of arcuate nucleus from the adjacent hypothalamic structures is not technically achievable [41]. Ablation of ARC becomes possible using genetic or chemical methods (e.g. MSG or more selectively neuropeptide Y (NPY)-saporin injection) and abolishes the ARC-PVN neuronic axis [20,97,98]. The clinical impact of that lesion concerns eating behavior and hyperphagia, obesity and IR [29,41,89,91].

BAT

The mentioned murine BAT located in the cervical–supraclavicular depot, plays a significant role on thermal energy production by expression of UCP1, which increases energy consumption in the mitochondria [99]. BAT ablation is achieved in transgenic models when a UCP1-specific diphtheria toxin A is expressed [27,41,89,99]. UCP1 deficiency reduces energy

expenditure and results in obesity and IR, which worsen after HFD [27,41,89]. In addition to UCP1 deficiency, β3 adrenergic receptor (beta3-AR) under-expression in BAT is associated to obesity through decreasing energy consumption [100].

Ovariectomy

Ovariectomy has been established as the mainstay procedure to create gonadectomy models, resulting in estradiol insufficiency. The phenotypic changes include hyperphagia, weight gain and adipose mass increase [27,88]. The exact pathophysiologic pathway after ovariectomy remains unclear; estrogen and leptin dysregulation has been underlined as the main mechanism, while a potential association of the role of NPY is also reported [87,101]. Despite the aforementioned advantages, ovariectomy lacks the normal, gradual decrease of sex hormones during menopause and the physiologic compensatory responses [27,41]. Additionally, lean mass in those rodent models is increased, unlike to human [102].

Summarizing, surgical models of obesity are less used nowadays, since they expose animal to stress and pain, given that there are less technically demanding/invasive alternative ways to mimic human pathology [1]. Moreover, mice are less appropriate due to their size (compared to other species) for the performance of operations [17]. Additionally, targeted genetic disruption of concrete regions or cells in brain is generally favoured over the mechanical approach, since the latter influences not only all neurons in the targeted area, but also neuronal connectivity between brain regions [28].

Genetic models of obesity

Mouse models of monogenic obesity

ob/ob mouse (*Lep^{ob}/Lep^{ob}* mouse or the "obese" mouse)

The first and more representative genetic mouse model of a single spontaneous mutation is the so-called "obese" or *ob/ob* mouse, which arose in Jackson Laboratories in 1949, discovered by Coleman et al., and led later to the discovery of leptin [28,29,41]. Leptin gene mutation (codon 105), which is seen in this model, is autosomal recessive (*ob/ob*) and is located on chromosome 6 and 7 in murine and human, respectively [27]. The homozygous mutant mice are typically of C57BL/6J strain. This animal presents an early pronounced obese phenotype as a first feature, characterized by hyperleptinemia, hyperphagia, followed by hyperinsulinemia, IR, severe obesity, mild diabetes and fatty liver [41]. However, unlike human obesity, *ob/ob* mice lack arterial hypertension and dyslipidemia even in the adolescence; on the contrary a HDL elevation has been described [27]. The animal exhibits hepatic steatosis, but the progression to steatohepatitis (NASH) does not occur. For this, it is necessary the exposure of the mouse to a toxic agent, unlike humans, in which progression is a natural consequence of the disease [103]. Additionally, these mice exhibit hypogonadism, hypothyroidism as well as GH insufficiency [28]. Interestingly, once the aforementioned leptin depletion is maintained on C57BL/KS strain, mice develop more severe diabetes with beta-cell complete loss and premature death [27,28]. Besides, in both *ob/ob* and *db/db* mouse models, adiponectin homolog osmotin seems to protect obesity/diabetes-induced NAFLD via activation of adiponectin receptors (R1/R2) and its downstream APPL1/PPAR-α/AMPK/SIRT1 pathways. Therefore, it could be a potential novel therapeutic agent against obesity/diabetes-induced NAFLD and other MetS disorders; novel osmotin decreases body weight, blood glucose and glycated hemoglobin, IR and hepatic glycogenesis, improves glucose tolerance and increases fatty acid oxidation and mitochondrial functions [104].

***db/db* mouse (the 'diabetic' mouse)**

In accordance to "obese" mouse, the nonsense, spontaneous mutation of gene decoding leptin receptor (*db/db*) gave birth to the "diabetic" mouse [28,29]. The gene is located on murine chromosome 4 [27] and on human chromosome 1 [105]. The predominant mouse strain in which this mutation is maintained is the C57BL/KS. The phenotype is similar to that of *ob/ob* mouse regarding obesity, hypogonadism and GH insufficiency [27,28]. However, more severe hyperglycemia and hyperinsulinemia are early (10th day) and progressively aggravate till 3 months. Later on, insulin levels fall accompanied by β-cells atrophy. Endpoint is an average level of blood glucose >400 mg/dl (22.2 mmol/L), which contributes to an early death, before vascular and retinal diabetic complications are identified. Once the leptin receptor mutation is produced in a C57BL/6J strain, lifespan is not affected and diabetic symptoms are only mild, whereas obesity remains remarkable [28]. Levels of leptin are increased due to the receptor mutation and subsequent leptin resistance. Thus exogenous administration of leptin, unlike to *ob/ob* mice, does not have any effect on weight reduction or other manifestations [41,90]. Recent data indicate that, in *db/db* mice, platycodin D (active compound of root of *Platycodon grandiflorum*, which possesses anti-obesity actions) improves obesity by AMPK-related reduction of adipogenic markers such as PPARγ, and increases thermogenic factors, suggesting that its anti-adipogenic and thermogenic actions are dependent on the mentioned AMPK (AMP-activated protein kinase) pathway activation [106]; and the observed increased steroid hormone production and adrenal steroidogenesis in the *db/db* model of obesity and T2DM, signify that steroid hormone dysfunction seems to be linked to obesity and T2DM development and correction of steroid abnormalities might offer new relative therapeutic approaches [107].

***s/s* mouse**

This mutant mouse is also leptin receptor deficient, but arose as GEM and is regarded as more specific than the "diabetic" mouse. Instead of ubiquitous leptin receptor dysfunction,

s/s mice are characterized by a mutation that disrupts the signal transducer and activator of transcription 3 (STAT3). They were developed by Bates at al. to clarify the role of individual leptin signals by provoking a knock-in mutation, which interrupted STAT3 pathway, a key molecule in intracellular leptin signaling [1,3,41]. Similarly to *db/db* mice, this mutation results in leptin resistance and subsequent obesity and diabetes. Homozygous *(s/s)* mice are obese and hyperphagic, but maintain fertility, normal body length and develop less profound hyperglycaemia compared to their counterparts *db/db*, probably due to leptin action via other intracellular pathways [41]. Energy restriction was able to normalize the glycemic control and IR in *s/s* mice [1].

Lethal yellow mutant mouse (Ay)

Agouti mutation mouse is one of the commonest and most successful models in obesity research. Although the mutation was known since the beginning of the last century, the model was created only in 1992. Agouti is a gene, responsible for controlling pigmentation and is expressed transiently in follicular melanocytes. It induces the production of yellow and red pheomelanin pigment with parallel inhibition of brown/black pigment [41,90,108]. Production of pheomelanin is regulated through antagonism of α-melanocyte–stimulating hormone (MSH) on melanocortin-1 receptor during 4th to 6th day of murine hair cycle. The lethal yellow mutation (A^y) is one of the five known dominant agouti mutations and results in an ectopic expression of agouti. Mutant mice appear different and complex phenotypes with more representative the yellow coat coloration, mature onset of obesity, T2DM, IR, hyperleptinemia, tumor susceptibility, infertility and increased linear growth (compared to “obese” mouse). Of note, several transgenic Agouti mice have been generated; the selective cutaneous expression did not lead to obesity, suggesting that the obesogenic role of agouti is tissue dependent [41,90,108]. Interestingly, in mice with the mentioned yellow mutation in the Agouti locus, metformin administration decreases body weight, restores the hypothalamic decreased activity of Akt

kinase (key component of leptin pathway), and normalizes glucose tolerance and the hypothalamic increased subtype 1B serotonin receptor [109]. Moreover, A^y mouse can serve as a novel and valuable preclinical model to investigate neurobehavioral complications correlated with obesity and T2DM [110].

Fat (*fat/fat* or Cpe fat) mouse

In 1973, another spontaneous gene mutation of inbred mice gave birth, manifesting obesity. Gene was named *fat*, its mutation was autosomal recessive and had no common components with *db* and *ob* genes. Later was demonstrated that mutation occurred within the gene of carboxypeptidase E (CPE) [111]. CPE is a peptide-processing enzyme, involved in cleaving numerous peptide precursors, including neuropeptides and hormones participated in appetite control and glucose metabolism. For instance, CPE contributes to conversion of pro-insulin to insulin in the periphery, and to cleavage of the pro-opiomelanocortine (POMC) in the CNS. Certain inactivating mutations of CPE result in inhibition of production of POMC derivatives, including α-MSH. It leads to to an imbalance between α-MSH and agouti-related protein activity at melanocortin receptors 3 and 4, thus promoting obesity [41].

"Fat mice" have a later onset of obesity than "diabetic" and "obese" mice, averagely at six to eight weeks [41,111,112]. By twelve weeks, the difference of body weight is profound, compared to wild-type littermates. A body weight of up to 70g can be seen in the first half year of life [111]. Of note, wild-type adult mice weigh 20-40g [113]. There are no observed gender differences in the rate of weight gain in mutant mice when compared to lean littermates. The excess weight in *fat/fat* mice is attributed to fat deposition to subcutaneous and visceral adipose tissue. Homozygous fat mutants become infertile, when obesity is established [41,111,112]. Male and female *fat/fat* mice develop hyperinsulinemia; however, male experience a transient hyperglycemia between 6 and 8 weeks, whereas female remain normoglycemic. Of note,

homozygous male mutant mice of C57BL/KS strain are particularly hyperglycemic, with reported blood glucose levels up to 600 mg/dL (33.3 mmol/L) [111].

Tubby (*tub/tub*) mouse

This mutation *(tub)* occurred spontaneously in a mouse of C57BL/6J genetic background [111]. The expression of *tub* is primarily in the central and peripheral nervous systems, with very low expression also found in the liver [111]. Its exact role remains largely unknown [41]. Homozygous mutant mice develop a late-onset obesity that progresses even slower than the previous models. Moreover, they exhibit severe deafness and blindness. Increase of body weight, mainly attributed to adipose tissue deposition, can be seen at about 12 weeks and adult mice might double their body weight compared to lean littermates, although tubby mice are not particularly hyperphagic. They also develop IR and hyperinsulinemia [111]. A mild hyperglycaemia can occur, but these mice do not develop typically an overt T2DM and they become infertile, when obesity is established [41,111]. Interestingly at 7–8 weeks, orexin, NPY and agouti-related peptide are found to be upregulated in homozygotes. Metabolic defects become apparent even before the onset of obesity [111]. Moreover, the occurrence of abnormal cholinergic/GABAergic vascular innervation in the ARC, indicates that alterations in this region, which includes neurons received information from the periphery and relays information about the energy status to other parts of the CNS, could be essential in the obese phenotype development in animals with an autosomal recessive mutation in the tub gene [114].

Summarizing, monogenic models have the advantage of developing more severe obesity with distinct phenotypes, which facilitates mainly studies of medications, since their targets and effects are well characterized. In addition, they require shorter duration, because long feeding periods to induce obesity are not needed [115]. Likewise, the genetic basis is homogeneous, the environmental factors are controlled, and the resulting variability is insignificant, thereby

permitting the use of smaller samples [69]. However, monogenic animals exhibit high mortality in certain strains, in addition to the necessity of sophisticated care of the animals, which makes the investigation more costly [69]. Although monogenic models have been particularly useful in obesity investigation, the common underlying mechanisms between monogenic obesity in humans and several related pathologies remains poorly understood [116]. Furthermore, monogenic obesity in humans represents only a small percentage, since common obesity in humans is mainly polygenic. Thus, the results of monogenic models may not be translated in the common human obesity.

Mouse models of polygenic obesity

New Zealand obese (NZO) mouse

This model of polygenic obesity also demonstrates T2DM exclusively in male mice. The phenotype is impressive; adipose tissue early represents more than 40% of total body weight. Moreover, NZO mice show hyperphagia and decreased physical activity [108]. Some of the implicated genes may be involved in the substrate utilization in the skeletal muscle and the triglyceride storage in adipocytes. Noteworthy, orthologs of at least some of these genes have been linked to human Mets [41]. Recent data in NZO mouse indicate that MetS is associated with augmented mitochondria and peroxisomal activity to cope with dyslipidemia and hypercholesterinemia driven hepatic lipid overflow in fatty liver development [117]; the cardiac phenotype in male NZO mice is connected with impaired cardiac energy function and signaling events [118]. *Etragonia tetragonoides* (Pall.) Kuntze, called New Zealand spinach, used in salad in Western countries, exhibits anti-obesity, anti-lipidemic and anti-hyperuricemic actions in HFD-induced NZO mice, partially explained by regulation of lipid-metabolism-related genes and proteins and decreased expression of xanthine oxidoreductase. The expression and activity

of the latter is associated with increased obesity and regulation of adipogenesis in adipose tissue [119].

Tsumura Suzuki obese diabetes (TSOD) mouse

Tsumura and Suzuki developed two new inbred strains by using a selection of obese and urine sugar positive colonies from mice with *ddY* background. Only the males of these strains are characterized by obesity with hyperinsulinemia, hyperglycemia and T2DM. Although hyperglycemia progressively becomes severe, diabetes remains respectively mild, as mice have increased β-cell mass and maintain sufficient insulin secretion to control glucose [26]. Diabetic nephropathy and neuropathy can be seen to older mice [108]. Interestingly, onset of NASH has been reported to TSOD mice and most of the older animals develop hepatocellular adenocarcinoma [120]. In addition, femur bone mass is lower in TSOD mice since hyperinsulinemia during pre-diabetic and established diabetic conditions increases bone resorption due to high bone turnover [121].

M16 Mouse

M16 mouse was created on an ICR genetic background (named from the distributing Institute of Cancer Research) after tactful selection of mice, which achieved a weight gain in 3-6 weeks; M16 mice is characterized as an outbred animal model to facilitate gene detection and pathway regulation monitoring early onset polygenic obesity and phenotypes of T2DM. Predominant phenotypes in this model, featuring obesity and diabesity, appear at a young age, thus closely mirroring current trends in human populations. They display, as compared to their wild-type littermates, hyperleptinemia, hyperinsulinemia and hyperphagia. Both sexes are also mild hyperglycemic [108,112].

Kuo Kondo (KK) mouse

Another polygenic model of obesity, which also demonstrates T2DM and was developed in Japan. Mice phenotype includes IR with accompanying hyperinsulinemia and hyperphagia as well as mild obesity at 2 months. Characteristic of this model is the late onset of obesity compared to the early onset of IR, implying that obesity is not the initial drive of IR. A variant of this model is the KKAy mouse, which was developed by transferring the mentioned lethal Ay gene (Ay) [108].

Rat models of monogenic obesity

The Zucker fatty rat (ZFR)

Lois Zucker, through an experimental study, introduced a rat model in obesity research in 1961 [122]. The ZFR is characterized by homozygous mutation of a recessive gene (*fa* gene; *fa/fa*) [123] on chromosome 5 expressing leptin receptors; the mutated receptor, although expressed, remains mainly intracellularly causing a functional deficit [108,122]. Primary obesity development at 3 weeks of life is the prominent phenotypic feature accompanied by secondary metabolic and hormonal deviations [41,108,122,124,125]. This animal produces leptin, though there is no action of the hormone in its receptor, resulting in hyperphagia, with hyperleptinemia. Apart from leptin, additional orexigenic hormones are also high in this model [125]. Adult ZFR exhibit 40% of their weight in the form of fat and peripheral and liver IR, though glucose levels are normal, without development of T2DM [126]. These findings resemble human exhibiting obesity and IR, but not T2DM [32]. Hypoglucagonemia, dyslipidemia and hypertension are other components of MetS in ZFR [122,124,125,127–129].

Sex hormones are also affected in both genders. Serum luteinizing hormone, follicle-stimulating hormone, prolactin and progesterone fluctuation is impaired in female *fa/fa* rats, thus affecting fertility [130,131]. Additional endocrine disorders, such as hypothyroidism and GH/insulin-like growth factor-1 (GH/IGF-1) axis disruption, are observed in all ZFR [41,89,132].

Interrupted glucose transportation seems to differentiate ZFR from a Zucker Diabetic Fatty rats (ZDF), thus predisposing to T2DM after HFD [41,68,89]. Data indicate that IR and the resultant increased reactive oxygen species (ROS) impair functional dilation in ZFR, increasing thromboxane receptor-mediated vasoconstriction, whereas the reduction of IR and postprandial hyperglycemia significantly improves vascular OS and function [133,134]

Wistar fatty rat (WFR)

In the early 1980s, coupling between Wistar Kyoto and Zucker *fa/fa* rats generated a monogenic leptin receptor depleted strain [135], which develops obesity and predisposes to MetS, IR, hyperinsulinemia, T2DM, hypertension and dyslipidemia (elevation of total cholesterol, low density lipoproteins cholesterol (LDL-C) and free fatty acids) [135,136]. Metabolic outcomes are diet-induced and male rats are more vulnerable to these abnormalities. Diabetic neuropathy and nephropathy affect the elderly WFR, thus rendering them appropriate model for human T2DM [137,138].

Additionally, studies of *in utero* HFD on WFR revealed genomic alterations in nucleic acids of hepatic cells and mitochondria affecting tissue IR and liver lipid profile [139–141]. Interestingly, a low-protein diet seems to prevent diabetes progression in WFRs by reducing fat weight, increasing plasma fibroblast growth factor (FGF) 21 and high-molecular-weight (HMW) adiponectin levels, as well as UCP1 expression in the BAT, thereby leading to suppression of diabetic nephropathy [142].

Koletsky rat (Obese Spontaneously Hypertensive Rat -SHROB)

SHROB were generated after cross-fertilization between a spontaneously hypertensive (SHR) female rat and a male Sprague–Dawley rat [31,132]. The prominent factor of obesity is a recessive mutation of leptin receptor gene (fa^k), causing a complete lack of the membrane-bound segments of all gene isoforms in mentioned [111], in contrast to the functional disorder of

leptin receptor in ZFR. Delayed weight gain begins after the 6th week, thus culminating at 750 to 1000g [31,41,132].

In addition to obesity, SHROB display IR combined with glucose intolerance, without T2DM development. Hypertension is more discrete than in ZFR and severe hypertriglyceridemia, mild hypercholesterolemia and NAFLD also occur. Abnormal protein excretion in urine after six months is early indicator of glomerulosclerosis and nephrosclerosis. These conditions are considered similar to human diabetic and hypertensive vasculopathy and nephropathy [31,41,132]. With regard to reproductive ability, SHROB exhibit infertility with high testosterone in male rats and follicular atresia as wells as fewer corpora lutea in female ones [143]. SHROB variants, such as obese homozygous (cp/cp) LA/N-cp and SHR/N-cp rats have been widely used in T2DM studies, reclaiming their metabolic similarities to human diabetic patients [132].

In view that prolactin-releasing peptide (PrRP) exhibits a potential to reduce food intake and improve obesity, recent data indicate that a new lipidized PrRP analog is efficient of improving glucose tolerance in obese SHROB rats by peripheral introduction, signifying that its effect on glucose metabolism is independent of leptin signaling and body weight lowering. Therefore this analog might be used as an agent with both anti-obesity and glucose-lowering properties [144].

Short life expectancy (10–12 months), high development cost, the rarity of leptin receptor mutation in human and harsh management of these mutations in rodents constitute disadvantages not only for SHROB, but also for all leptin receptor-deficient rats [41,132].

DS obese rat

One of the most recently introduced rodent models for obesity, the DahlS.Z-Lepr^{fa}/Lepr^{fa} rat strain, resulted after crossing the salt-sensitive Dahl rats and ZFR. Except for weight gain, additional features constitute DS obese rats suitable for MetS studies: visceral and

subcutaneous adiposity, NAFLD, hypertension, elevated LDL-C to high density lipoprotein-cholesterol (HDL-C) ratio, premature heart aging and diastolic impairment, renal dysfunction, and urine protein excretion [145,146]. Interestingly, recent data indicate that the anti-inflammatory properties of atorvastatin on the cardiac and adipose tissues are attributable partially to the mentioned increased AMPK activity and decreased of nuclear factor NF-κB activity in DS obese rat model of MetS. [147].

The GH-deficient dwarf (dw/dw) rat

A spontaneous mutation of GH gene results in lower GH production and systematic release, thus provoking various manifestations. GH-deficient dwarf rats experience adipose tissue increase and impaired lipolysis. Some data indicate that the adiposity profile in dw/dw rats offers evidence of developmental abdominal leanness and hypoleptinemia in the context of profound GH deficiency. The underlying mechanisms of this puzzling leanness are yet to be recognized although do not seem to be the direct result of action in either GH signaling or energy intake. Identification of the genetic defect responsible for the dw/dw phenotype could discover a novel mechanism involving in the regulation of adiposity, and potentially new treatments. [148].

Rat models of polygenic obesity

Wistar Ottawa Karlsburg W (WOKW) Rat

WOKW rat was generated by crossing outbred Wistar rat and BioBreeding diabetic rat, by inheriting RT1 haplotype of the major histocompatibility complex [132]. The predominant manifestation of this inbred model is IR, in addition to obesity hyperlipidemia, hypertension, impaired glucose tolerance, hyperinsulinemia and enhanced autophagy in IR adipose tissue [149–151]. Specifically, up-regulated autophagy in obese WOKW rats contributes to the regulation of visceral adipose tissue function and involves a changed balance between pro-inflammatory and

protective adipokine expression, thereby suggesting that adipose tissue autophagy activation seems to protect against adipocyte apoptosis under conditions of obesity-related MetS in WOKW rats; autophagy may act as a key protective rather than as destructive feature [152]. Polygenic regulation of MetS in WOKW rats is considered as an advantage in MetS studies [22,150]. Moreover, elderly WOKW rats predisposed to coronary artery disease, thus reflecting human results of MetS [153].

Sprague-Dawley rats

Sprague-Dawley rats, in addition to Wistar rats, belong to the most commonly used rat models in diet surveys, as they offer easier manipulation and durability [31,38]. These outbred rodents reflect the distribution of human population in obesity development; HFD provokes weight gain to many of these rats [3,29,41,112]. Exhibition to high carbohydrate diets, such as fructose, could additionally cause hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, hypertension and IR [31]. Recently, Ichimura et al. [154] showed that high fat and cholesterol diet induced NASH and hepatic fibrosis in Sprague-Dawley rats. Interestingly, DIO Sprague-Dawley rats display early post-weaning indicators of hippocampal inflammation, lipid peroxidation, reduced neural progenitor cell proliferation and defective hippocampal dependent memory by early adulthood, signifying that inherent metabolic alterations predispose this DIO strain to cognitive deficit prior to exposure to HFD and manifestation of obesity [155]. In addition, maternal and post-weaning HFD settings could disturb hypothalamic neuronal signal irrelevantly, which is essential for leptin regulation of energy homeostasis and induce the risk of Sprague-Dawley rat offsprings to future metabolic disorders [156]. Moreover, fecal microbiota transplantation appears to alleviate gut transit in the HFD-fed Sprague Dawley rats by regulating the serotonin biosynthesis, thus relieving gastrointestinal dysmotility (GID) and providing a perspective on the association between obesity and GID, as well as on the requirement for gut microbiota-based therapy for the alleviation of GID [157].

Nile rat (African grass rat; *Arvicanthis niloticus*), Israeli sand rat (*Psammomys obesus*)

Both Nile and Israeli sand rats were established for the purposes of MetS studies after feeding them with a chow diet. These wild rodents remain with normal weight in their natural habitat, whereas they develop obesity and T2DM in captivity - laboratory. Specifically, diabetes development and progression is very fast in the Israeli sand rats reaching the irreversible hypoinsulinemic disease stage, in which a marked reduction of β-cell mass is apparent, within 4-6 weeks of high caloric diet [158]. Metabolic disorders comprise hypertension, dyslipidemia, NAFLD and abdominal adiposity, thus resembling human obesity and glucose intolerance. The fundamental disimilarity is that the chow diet induces MetS, instead of high caloric input in human patients [68].

The Otsuka Long-Evans Tokushima Fatty (OLETF) Rat

OLETF rats resist to cholecystokinin peptide stimulus to food intake control, owing to the absence of its pancreatic receptor [30,132]. An additional mechanism suggests the overexpression of NPY in the dorsomedial hypothalamus, thus inducing hyperphagia and impairing management of the energy balance [159]. The phenotypic features of these models are hyperphagia, late onset obesity and T2DM (after 20 weeks), thus increasing weight by about 1.5 times compared with the lean control and exhibiting glucose intolerance by week 24 [68]. Additional MetS components of these rodents are late onset hypertension, the isolated triglyceride increase, but for a slight elevation of serum cholesterol and NAFLD [68,132]. OLEFT rats consist an important tool for studies of target-organ lesions due to MetS, as they manifest heart failure and glomerulosclerosis in adulthood [68]. Nevertheless, weight loss was observed when OLETF rats access the running wheel and, depending on experimental conditions, weight preservation was achievable after exercise cessation. Current studies on OLETF target to illuminate NPY role and regulation, in order to clarify and suggest treatment options against

obesity [101]. Interestingly, cannabinoid 1 (CB1) receptor antagonist, rimonabant, seems to reverse NASH and its related features of MetS, by ameliorating hepatic fat infiltration, inflammation, cellular death and mRNA expression of proinflammation and fibrosis genes in a OLETF rat model of severely uncontrolled diabetes, implying the potential of pharmacological CB1 receptor blockade as a therapeutic regimen in the progression of NASH [160].

One basic advantage of polygenic models as already discussed, is that they recapitulate better the human obesity and its energetic precursors. However its genetic basis is rather complex implicating many genes, rather than single gene mutations, which could simplify the research and reaching conclusions [1,3].

Concluding remarks

The animal models can be considerably different from each other owing to the methods introduced to induce obesity. For instance, when compared with monogenic models, the DIO models could display less hyperphagia and several obese animal models do not exhibit increased fasting glucose levels and diabetes, despite similar levels of IR. Therefore, it is important to be aware of these characteristics when choosing obese animal model. Typical paradigms of potential non-specific impacts (such as hyperglycemia and hyperleptinemia) on innate immune responses from these different baseline parameters include the the fasting hyperglycemia in Zucker diabetic fatty rats vs. normal fasting glucose in obese Zucker rats, and the lack of leptin in *ob/ob* mice vs. high leptin in *db/db* mice. Likewise, investigators ought to be aware of the effect of aging on cardiovascular and immune systems in the obese animals models [161]. Moreover, MetS parameters, including hyperlipidemia and hyperglycemia, frequently occur in obese models and seem to play an essential role in the development of chronic inflammation and CVD.

Within this essay, we provided information regarding the most common mice and rat models of obesity and commented their advantages and limitations. Rodent models of obesity can recapitulate in a satisfactory manner the human pathology. The recent advantages in obesity pathophysiology can partly be attributed to the aforementioned preclinical studies with animal models. Although organoids become lately available and useful for tactfully chosen scientific questions, they are not able to fully replace the current cornerstone of basic research, namely mammalian models, since the latter better mimic the human, including physiology, nutrition, exercise, CNS control of obesity and body fat distribution. Moreover, it should be emphasized that a "wise" choice of the proper model of obesity would allow researchers reach validated and translational conclusions. Another substantial aspect is animal ethics and welfare, which should not be underestimated. Laboratory rodents are exposed to the chronic stress of manipulations and obesity with all their consequences and later on they are sacrificed. Principles which should be considered in experiments with laboratory animals include the so-called 3Rs (replacement, reduction and refinement), originally introduced in 1959, with the later addition of 2Rs (robustness and reproducibility) [162]. Wide-known guidelines which describe precisely how animal welfare is achieved and how stress is avoided are the ARRIVE ones [162]. With these measures can humankind express its gratitude towards animals, which have largely contributed to substantial insights into the etiology and treatment of human diseases, including obesity.

Abbreviations: ARC; arcuate nucleus, BAT; brown adipose tissue, CPE; Carboxypeptidase E, DS; DahlS.Z-*Lepr*^{fa}/*Lepr*^{fa}, GH; growth hormone, HFD; high fat diet, HSD; high sugar diet, OLETF; Otsuka Long-Evans Tokushima Fatty rats, PVN; paraventricular nucleus, VMH; ventromedial hypothalamus, WOKW; Wistar Ottawa Karlsburg W

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Table. Features and metabolic components of Obesity rodent models

	Model Name	Disorder	Hyperphagia	IR	Hyperglycemia	T2DM	Hypertension	Dyslipidemia	NAFLD
Monogenic Mutated Models	*ob/ob* mouse	*Lep*^{ob} mutation	+	+	+	±	-	-	+
	db/db mouse	*db* leptin receptor mutation	+	+	+	+	-	+	+
	s/s mouse	STAT3 mutation	+	+	±	-	-	-	+
	Ay Lethal yellow mutant mouse	Agouti mutation	+	+	+	+	-	+	-
	fat/fat or Cpe fat mouse	carboxypeptidase E mutation	+	+	+	-	-	±	-
	Tubby mouse	*tub* mutation	-	+	+	-	-	-	-
	ZFR	*fa* leptin receptor mutation	+	+	+	-	+	+	+
	WFR	leptin receptor depletion	+	+	+	+	+	+	+
	Koletsky rat	*fa*^{k} leptin receptor mutation	+	+	+	-	+	+	+
	DS obese rat	Lepr^{fa}/Lepr^{fa} mutation	+	+	-	-	+	+	+
	The GH-deficient dwarf rat	GH gene mutation	-	+	-	-	-	-	+
Polygenic models	New Zealand obese mouse	male sex dependent	+	+	+	+	+	+	-
	Tsumura Suzuki mouse	male sex dependent	+	+	+	+	+	+	+
	M16 mouse	ICR based mouse	+	+	+	-	-	-	-
	KK mouse	diet dependent	+	+	+	+	-	-	-
	WOKW Rat	RT1 haplotype inheritance	+	+	+	-	+	+	-
	Sprague Dawley rats	diet dependent	+	+	+	-	+	+	+
	Nile rat, Israeli sand rat	diet dependent	+	+	+	+	+	+	+

	OLETF Rat	cholecystokinin resistance, NPY overexpression	+	+	+	+	+	+	+
Surgical Models	VMH lesion	parasympathetic stimulations predominance	+	+	+	+	+	+	-
	PVN lesion		+	±	±	-	+	-	-
	ARC lesion	ARC-PVN axis abolition	+	+	±	-	-	-	-
	BAT lesion	UCP1 deficiency	-	-	-	-	-	-	-
	Ovariectomy	estradiol insufficiency	+	-	-	-	+	+	+

ARC; arcuate nucleus, BAT; brown adipose tissue, DS; DahlS.Z-Lepr^{fa}/Lepr^{fa}, GH; growth hormone, IR; insulin resistance, KK; Kuo Kondo, NAFLD; nonalcoholic fatty liver disease, NPY; neuropeptide Y, OLETF; Otsuka Long-Evans Tokushima Fatty rats, PVN; paraventricular nucleus, STAT3; signal transducer and activator of transcription 3,T2DM; diabetes mellitus type 2, UCP1; uncoupling protein 1, VMH; ventromedial hypothalamus, WFR; Wistar fatty rats, WOKW; Wistar Ottawa Karlsburg W, ZFR; Zucker fatty rat

Table 2. Basic features of obesity inducing diets

	Meal frequency	Portion	Caloric source	Limitations
High Fat Diet (HFD)	Normal	Normal	45-60% animal fat	High cost, model, age and sex dependent
Cafeteria diets	Increased	Increased	High fat and/or high sugar nutrition	Difficult calculations of energy and macronutrient intake, lack of standarization
Fat or sugar choice	Increased	Normal	Free-choice of "high-fat or high-sugar" diet	
Meal feeding	Unlimited	Unlimited	The most representantive: *ad libitum* model,offering to the subjects an unlimited access to a specific HFD	Multi-variances dependent, Plethora of subtypes
Binge-type feeding	Scheduled	Increased	Flavorful meals, "Rewarding" pattern	Result based on rodent's susceptibility